PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

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Applicant's or agent's file reference	FOR FURTHER ACTION	See Form PCT/IPEA/416
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International application No. PCT/EP2004/013398	International filing date *(day/month/year)* 26.11.2004	Priority date *(day/month/year)* 09.12.2003
International Patent Classification (IPC) or national classification and IPC C12Q1/68		
Applicant NANOGEN RECOGNOMICS GMBH et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 9 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 2 sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
07.07.2005	10.02.2006
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Cornelis, K Telephone No. +31 70 340-8957

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/EP2004/013398

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-7	as originally filed

Sequence listings part of the description, Pages

1-4	as originally filed

Claims, Numbers

8-10	as originally filed
1-7	received on 17.11.2005 with letter of 14.11.2005

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☒ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☒ the claims, Nos. 1-6
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-5,9,10
	No:	Claims	6-8
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-10
Industrial applicability (IA)	Yes:	Claims	1-10
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed

   ☒ filed together with the international application in computer readable form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment on

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

Section I: Basis

The subject matter of claim 1 does not fulfill the requirements of Article 34(2)b PCT. The application does not disclose a method to detect malignancy of melanoma cells wherein **an oligonucleotide probe** comprising exon 15 is hybridised with a wild typ **reporter** and a mutant reporter. This method does not use any nucleic acid derived from tissue, it could therefore not detect the malignancy of melanoma cells. A claim relating to "a method for the detection of the malignancy of melanoma cells, wherein a a sample comprising DNA comprising exon 15 of the BRAF gene or a part thereof comprising codon 599 or the counterstrands thereto, is hybridised with a labelled wild type reporter..." would be within the disclosure of the filed application.

Dependent claims 2-6 include embodiments outside of the scope of claim 1. E.g. claim 2 refers to the use of an oligonucleotide comprising a sequence with SEQ ID NO 1 which is much longer than either SEQ ID Nos 5 or 6, and to parts or variants comprising codon 599.

Section V: Novelty and Inventive step

The following opinion was made disregarding the amended claims 1-6, the amended claim **7 corresponds to the original claim 9**. Therefore, the following opinion is based on the claims as originally filed, and on the opinion on new claim 7 is the same as for original claim 9.

The following documents (D) are referred to in this communication; the numbering will be adhered to in the rest of the procedure:

D1: DONG JIANLI ET AL: 'BRAF oncogenic mutations correlate with progression rather than initiation of human melanoma.' CANCER RESEARCH. UNITED STATES 15 JUL 2003, vol. 63, no. 14, 15 July 2003 (2003-07-15), pages 3883-3885, XP002278827 ISSN: 0008-5472

D2: WO 02/090512 A (CHEW ANNE ;KAZEMI AMIR (US); KOSHY BEENA (US); GENAISSANCE PHARMAC) 14 November 2002 (2002-11-14)

D3: DATABASE EMBL [Online] 15 June 1992 (1992-06-15) SITHANANDAM ET AL.: 'Homo sapiens B-raf protein (BRAF) mRNA, complete cds' retrieved from EBI Database accession no. hsbraf3 XP002278828

1 NOVELTY (Article 33(2) PCT)

1.1 Independent claim 6, dependent claims 7 and 8

The document D1 discloses (the references in parentheses applying to this document): a method to detect the malignancy of melanoma cells wherein the presence of a mutation in codon 599 in exon 15 of the BRAF gene or a part thereof comprising codon 599 is determined (Abstract; Results and Discussion: 3rd paragraph), wherein the presence of a mutation in codon 599, which leads to a replacemnt of valine into glutamic acid or aspartic acid, is determined (Results and Discussion: 2nd paragraph) by sequencing (Materials and Methods: PCR and Direct Sequencing).
The subject-matter of claims 6-8 is therefore not new (Article 33 (2) PCT).

2 INVENTIVE STEP (Article 33(3) PCT)

2.1 Independent claim 1

2.1.1
Document D1, which is considered to represent the most relevant state of the art, discloses the use of oligonuceotides for sequencing a part of exon 15 of the BRAF gene comprising codon 599 for detect the malignancy of melanoma cells.
The subject-matter of claim 1 differs from D1 in that it uses an oligonucleotide probe wich comprises exon 15 or a part thereof which comprises codon 599. The technical effect of this difference is that it provides another detection method for the mutation at codon 599 of the BRAF gene.

2.1.2
The problem to be solved by the present invention may therefore be regarded as the provision of an alternative detection method for a mutation in codon 599 of BRAF. The solution is to use an oligonucleotide probe which comprises codon 599.

2.1.3
The solution proposed in claim 1 of the present application cannot be considered as involving an inventive step (Article 33(3) PCT) for the following reasons:
The use of oligonucleotide probes which comprise the site in which alleles differ from each other to determine the genotype is well known in the art. It is for e.g. used in D2 (p 17, line 25-35) to detect a polymorphism either before or after amplification in the NNMT gene which is

implicated in a cancer related disease. Thus the person skilled in the art who wanted to develop an alternative detection method for the mutation in codon 599 of BRAF would easily encounter the alternative of claim 1 in the prior art.

2.2 Claims 2-5

Dependent claims 2-5 do not appear to contain any additional features which, in combination with the features of any claim to which they refer, meet the requirements of the EPC with respect to inventive step, the reasons being as follows:

D1 mentions the accesion number of the cDNA sequence of BRAF (which can also be found in D3), which has 100% identity with the sequence in SEQ ID NO 1. The use of an oligonucleotide which has a sequence comprising a part of SEQ ID NO 1 which comprises codon 599 or an allelic variant is normal design procedure. Oligonucleotides with a sequence comprising SEQ ID NO 5 or 6 are merely a selection of all the possible oligonucleotides which could be used as a probe to detect either wild type or a mutant codon 599. Therefore claims 2 and 5 cannot be considered inventive.

D1 discloses that codon 599 can bear a mutation and can encode (amongst others) valine, glutamic acid or aspartic acid. The features of claims 3 and 4 are already disclosed in closest prior art document D1 and therefore the subject matter of claims 3 and 4 cannot be considered inventive.

2.3 Independent claims 9 and 10

The difference between claim 9 and D1 is that D1 does not use reporter oligonucleotides that comprise a sequence with SEQ ID NO 5 or 6. However, D2 discloses reporter oligonucleotides which overlap with the mutation which has to be detected (page 17 lines 25-35). Oligonucleotides with a sequence comprising SEQ ID NO 5 or 6 are merely a selection of all the possible oligonucleotides which could be used as a probe to detect either wild type or a mutant codon 599. Therefore claims 9 and 10 cannot be considered inventive.

3 Further remarks

Should claim 1 be formulated as "a method for the detection of the malignancy of melanoma cells, wherein a sample comprising DNA comprising exon 15 of the BRAF gene or a part

thereof comprising codon 599 or the counterstrands thereto, is hybridised with a labelled wild type reporter comprising a sequence according to SEQ ID NO 5 and a differently labelled mutant reporter comprising a sequence according to SEQ ID NO 6 and wherein the intensity ration of the signals derived from the hybridised reporters is determined" the subject matter of the claim would still not be considered inventive.

The reasons for this would be as follows:

The closest prior art document would be D1, which discloses the use of oligonuceotides for sequencing a part of exon 15 of the BRAF gene comprising codon 599 for detect the malignancy of melanoma cells.

The subject-matter of claim 1 would differ from D1 in that it uses labelled probes wich comprise codon 599 in the wild type and mutant variant. The technical effect of this difference is that it provides another detection method for the mutation at codon 599 of the BRAF gene. There is no evidence in the application that using reporter probes which comprise or consist of a DNA with a sequence as in SEQ ID NO 5 and 6, results in an improved detection of the mutation, neither that determining the intensity signals in a ratio between the 2 reporters, has an advantageous effect compared to sequencing, as in D1.

The problem to be solved would therefore be regarded as the provision of an alternative detection method for a mutation in codon 599 of BRAF. The solution is to use 2 oligonucleotide probes which comprise codon 599 and are differentially labelled. The determination of the ratio between the 2 signals from the is a practical measure of the detection.

The solution would not be considered as involving an inventive step (Article 33(3) PCT) for the following reasons:

The use of labelled oligonucleotide probes which comprise the site in which alleles differ from each other to determine the genotype is well known in the art. It is for e.g. used in D2 (p 17, line 25-35; page 16 line 30--page 17, line 5) to detect a polymorphism either before or after amplification in the NNMT gene which is implicated in a cancer related disease. Also the determination of a ratio between signals is used in D2, for a similar purpose. Thus the person skilled in the art who wanted to develop an alternative detection method for the mutation in codon 599 of BRAF would encounter several alternatives in the prior art, and choose one of those without inventive skill.

Claims:

1. A method for the detection of the malignancy of melanoma cells , wherein an oligonucleotide probe comprising exon 15 of the BRAF gene or a part thereof comprising codon 599 or the counterstrands thereto is hybridised with a labelled wildtyp reporter comprising a sequence according to **Seq. ID No. 5** and a different labelled mutant reporter comprising a sequence according to **Seq. ID No. 6** or a sequence complementary to **Seq. ID No. 5** or **Seq. ID No. 6** or a sequence with a homology of over 80% to said sequences and wherein the intensity ration of the signals derived from the hybridised reporters is determined.

2. A method according to claim 1 wherein an oligonucleotide comprising the sequence **Seq. ID No. 1** or an oligonucleotide comprising a sequence complementary to **Seq ID No. 1** or a part of said sequences comprising codon 599 or an allelic variant thereof is used for the detection of the malignancy of melanoma cells.

3. A method according to claim 1 or 2 wherein the used oligonucleotide probe comprising codon 599 is bearing a mutation.

4. A method according to claim 1 or 2 wherein codon 599 of the used oligonucleotide probe codes for an amino acid selected from the group consisting of valine (Val, V), glutamic acid (Glu, E) and aspartic acid (Asp, D).

5. A method according to any one of claim 1 to 4 wherein the presence of a mutation in codon 599 in exon 15 of the BRAF gene or a part thereof comprinsing codon 599 is determined.

6. A method according to claim 5 wherein the presence of a mutation in codon 599 leading to a replacement of valine (wildtyp) into glutamic acid (Glu, E) or aspartic acid (Asp, D) is determined.

7. The use of reporter oligonucleotides comprising a sequence **Seq. ID No. 5** or **Seq. ID No. 6** or a sequence with an homology of over 80% to **Seq. ID No. 5** or **Seq. ID No. 6** or a sequence complementary to said sequences for the determination of the malignancy of melanoma cells.